CONFIDENTIAL

CIT

SPONSOR
Elf Atochem S.A.
Cours Michelet
La Défense 10
92091 Paris-la-Défense CEDEX
France

TEST SUBSTANCE
[redacted]

STUDY TITLE
ACUTE EYE IRRITATION IN RABBITS

STUDY DIRECTOR
Xavier Manciaux

STUDY COMPLETION DATE
9 December 1999

PERFORMING LABORATORY
CIT
Centre International de Toxicologie
BP 563 - 27005 Evreux - France

Company Sanitized. Does not contain TSCA CBI

LABORATORY STUDY NUMBER
18750 TAL

CONTENTS

STATEMENT OF THE STUDY DIRECTOR

The study was performed in compliance with the principles of Good Laboratory Practice as described in:

- OECD Principles on Good Laboratory Practice (as revised in 1997), ENV/MC/CHEM (98) 17.
- Décret N° 90-206 du 7 mars 1990 concernant les Bonnes Pratiques de Laboratoire (Journal Officiel du 9 mars 1990), Ministère de l'Industrie et de l'Aménagement du Territoire.
- Council Directive 87/18/EEC of 18 December 1986 on the harmonization of laws, regulations or administrative provisions relating to the application of the Principles of Good Laboratory Practice and the verification of their applications for tests on chemical substances (OJ No. L 15 of 17.1.87).

I declare that this report constitutes a true and faithful record of the procedures undertaken and the results obtained during the performance of the study.

This study was performed at CIT, Centre International de Toxicologie, BP 563, 27005 Evreux, France.

Toxicology

X. Manciaux — Date: 9 December 1999
Study Director
Doctor of Pharmacy

OTHER SCIENTISTS INVOLVED IN THIS STUDY

For Pharmacy: P.O. Guillaumat
Doctor of Pharmacy

For Toxicology: C. Pelcot
Study Supervisor

STATEMENT OF QUALITY ASSURANCE UNIT

Type of inspections	Dates		
	Inspections	Reported to Study Director (*)	Reported to Management (*)
Protocol	4 June 1999	4 June 1999	4 June 1999
Report	10 November 1999	7 December 1999	7 December 1999

In addition to the above-mentioned inspections, at about the same time as the study described in the present report, "process-based" and routine facility inspections of critical procedures relevant to this study type were also made by the Quality Assurance Unit.
The findings of these inspections were reported to the Study Director and to CIT Management.

The inspections were performed in compliance with CIT Quality Assurance Unit procedures and the Good Laboratory Practice.

The reported methods and procedures were found to describe those used and the results to constitute an accurate and complete reflection of the study raw data.

L. Valette-Talbi Date: 9 December 1999
Doctor of Biochemistry
Head of Quality Assurance Unit
and Scientific Archives

(*) The dates indicated correspond to the dates of signature of audit reports by Study Director and Management.

SUMMARY

At the request of Elf Atochem S.A., Paris-la-Défense, France, the potential of the test substance [redacted] to induce ocular irritation was evaluated in rabbits according to OECD (No. 405, 24th February 1987) and EC (92/69/EEC, B.5, 31st July 1992) guidelines.
The study was conducted in compliance with the principles of Good Laboratory Practice Regulations.

Methods

The study design was established according to available information on the test substance and the above guidelines.

As no irritant effects were anticipated, a single dose of 0.1 ml of the undiluted test substance was instilled into the conjunctival sac of the left eye of three male New Zealand White rabbits. The right eye was not treated and served as control.
The eyes were not rinsed after administration of the test substance.

Ocular reactions were observed approximately 1 hour, 24, 48 and 72 hours after the administration.

The mean values of the scores for chemosis, redness of the conjunctiva, iris lesions and corneal opacity were calculated for each animal.

Results

No ocular reactions were observed during the study.

Mean scores calculated for each animal over 24, 48 and 72 hours were 0.0, 0.0 and 0.0 for chemosis, 0.0, 0.0 and 0.0 for redness of the conjunctiva, 0.0, 0.0 and 0.0 for iris lesions and 0.0, 0.0 and 0.0 for corneal opacity.

Conclusion

Under our experimental conditions, the test substance [redacted] is non-irritant when administered by ocular route to rabbits.

According to the classification criteria laid down in Commission Directive 93/21/EEC (27th April 1993) adapting to technical progress for the eighteenth time Council Directive 67/548/EEC, the test substance is considered non-irritant.

RESUME

A la demande de Elf Atochem S.A., Paris-la-Défense, France, les propriétés irritantes oculaires du produit [redacted] après application unique chez le Lapin ont été évaluées selon les lignes directrices de l'OCDE (n° 405, 24 février 1987) et de la CEE (92/69/EEC, B.5, 31 juillet 1992).
L'étude a été réalisée conformément aux règles de Bonnes Pratiques de Laboratoire.

Méthode

L'étude a été réalisée selon les informations disponibles sur le produit et les lignes directrices mentionnées ci-dessus.

Aucun effet irritant n'étant supposé, une dose unique de 0,1 ml de produit non dilué a été instillée dans le cul de sac conjonctival de l'oeil gauche de 3 lapins mâles New Zealand White. L'oeil droit n'a pas été traité et a servi de témoin.
Aucun rinçage des yeux n'a été réalisé après l'administration du produit.

Les réactions oculaires ont été observées environ 1 heure, 24, 48 et 72 heures après l'administration.

La moyenne des scores pour le chémosis, la rougeur de la conjonctive, les lésions de l'iris et l'opacité de la cornée a été calculée pour chaque animal.

Résultats

Aucune réaction oculaire n'est observée pendant l'étude.

La moyenne des scores enregistrés pour chaque animal après 24, 48 et 72 heures est de 0,0 ; 0,0 et 0,0 pour le chémosis, 0,0 ; 0,0 et 0,0 pour la rougeur de la conjonctive, 0,0 ; 0,0 et 0,0 pour les lésions de l'iris et 0,0 ; 0,0 et 0,0 pour l'opacité cornéenne.

Conclusion

Dans nos conditions expérimentales, le produit [redacted] est considéré non irritant par voie oculaire chez le Lapin.

Selon les critères de classification décrits dans la Directive 93/21/CEE (27 avril 1993) portant dix-huitième adaptation au progrès technique de la Directive 67/548/CEE, le produit est considéré non irritant.

1. INTRODUCTION

The objective of this study was to evaluate the potential of the test substance [redacted] to induce irritation following a single ocular administration in rabbits.

In the assessment of the toxic characteristics of a test substance, determination of the irritant effects on the eyes of mammals is an important initial step. Information derived from this test serves to indicate the possible hazards likely to arise from exposure of the eyes, and associated mucous membranes, to the test substance.

This study was conducted in compliance with:
. OECD guideline No. 405, 24th February 1987,
. EC Directive No. 92/69/EEC, B.5, 31st July 1992.

2. MATERIALS AND METHODS

2.1 TEST SUBSTANCE

2.1.1 Identification

The test substance [redacted] used in the study was supplied by Elf Atochem S.A.

The test substance was identified as follows:
. name:
- protocol and labelling: [redacted]
. batch number:
- protocol and labelling: [redacted]

Elf Atochem filing number: 1392/99
. description: amber liquid
. container: one smoked glass flask
. date of receipt: 5 May 1999
. storage conditions: at room temperature and protected from light
. expiry date: May 2000.

Data relating to the characterization of the test substance are documented in a test article description and an analytical certificate (presented in appendix 1) provided by the Sponsor.

The pH of the test substance, as mentioned on the test article description, was 5 ± 1.

2.1.2 Formulation procedure

The test substance was used undiluted.

2.2 TEST SYSTEM

2.2.1 Animals

Sex, species, strain: male New Zealand White rabbits.
Reason for this choice: species generally accepted by regulatory authorities for this type of study.
Breeder: Elevage des Dombes, 01400 Châtillon-sur-Chalaronne, France.
Number of animals: three animals were used, as recommended by the international guidelines.
Identification: the animals were identified individually with a metal ear tag.
Weight: on the day of treatment, the animals had a mean body weight ± standard deviation of 3.0 ± 0.1 kg.
Acclimatization: at least 5 days before the beginning of the study.

2.2.2 Environmental conditions

The conditions in the animal room were set as follows:
. temperature: 18 ± 3°C
. relative humidity: 30 to 70%
. light/dark cycle: 12 h/12 h
. ventilation: approximately 12 cycles/hour of filtered, non-recycled air.
The temperature and relative humidity were under continuous control and recording. The records were checked daily and filed. In addition to these daily checks, the housing conditions and corresponding instrumentation and equipment were verified and calibrated at regular intervals.
The animals were housed individually in polystyrene cages (48.2 cm x 58 cm x 36.5 cm).
Each cage was equipped with a food container and a water bottle.

2.2.3 Food and water

During the study, the animals had free access to 112 C pelleted diet (UAR, 91360 Villemoisson-sur-Orge, France).
Each batch of food was analysed by the supplier for composition and contaminant levels.
The diet formula is presented in appendix 2.

Drinking water filtered by a FG Millipore membrane (0.22 micron) was provided *ad libitum*.
Bacteriological and chemical analyses of the water and diet, including the detection of possible contaminants (pesticides, heavy metals and nitrosamines), are performed regularly by external laboratories.
The results of these analyses are archived at CIT.

No contaminants were known to have been present in the diet or drinking water at levels which may be expected to have interfered with or prejudiced the outcome of the study.

2.3 TREATMENT

2.3.1 Selection of the animals

The day before treatment, the eyes of each animal were examined in order to use only animals without any signs of ocular lesions. Animals showing signs of ocular irritation, ocular defects or pre-existing corneal injury were not used.

2.3.2 Study design

The study design was established according to available information on the test substance and according to the OECD (No. 405) and EC (92/69/EEC, B.5) guidelines.

As no irritant effects were anticipated, the test substance was evaluated in three animals.

2.3.3 Administration of the test substance

A single dose of 0.1 ml of the undiluted test substance was instilled into the conjunctival sac of the left eye after gently pulling the lower lid away from the eyeball.

The lower and upper eyelids were held together for about one second to avoid any loss of test substance. The right eye, which remained untreated, served as control.

The eyes were not rinsed after administration of the test substance.

2.3.4 Chronology of the study

Animal number	Date of treatment (day 1)	End of the observation period
900	15 July 1999	18 July 1999
47	15 July 1999	18 July 1999
48	15 July 1999	18 July 1999

2.4 OCULAR EXAMINATIONS

The eyes were examined approximately 1 hour, 24, 48 and 72 hours after administration of the test substance.

Following the OECD and EC guidelines:
. when there was no evidence of irritation after 72 hours, the study was ended.
. when there was persistent ocular irritation after 72 hours, the observation period was extended to a maximum of 21 days (until day 22) in order to determine the progress of the lesions and their reversibility.
. when severe irritant effects were observed, the animals were killed on humane grounds.

Any change in the animals' behaviour was noted.

2.5 DESCRIPTION AND EVALUATION OF OCULAR REACTIONS

Ocular reactions were evaluated for each animal according to the following numerical scale:

2.5.1 Conjunctival lesions and discharge

Chemosis (lids and/or nictitating membranes)

. no swelling 0
. any swelling above normal (includes nictitating membranes) 1
. obvious swelling with partial eversion of lids 2
. swelling with lids about half-closed 3
. swelling with lids more than half-closed 4

Redness (refers to palpebral and bulbar conjunctivae, cornea and iris)

. blood vessels normal 0
. a number of blood vessels definitely hyperemic (injected) 1
. diffuse, crimson colour, individual vessels not easily discernible 2
. diffuse, beefy red 3

Discharge

. absence of discharge 0
. slight discharge (does not include small amounts normally found in inner canthus) 1
. discharge with moistening of lids and hairs adjacent to lids 2
. discharge with moistening of lids and hairs on wide area around the eye 3

2.5.2 Iris lesions

. normal 0
. markedly deepened rugae, congestion, swelling, moderate circum-corneal hyperemia, or injection, any of these or combination of any thereof, iris still reacting to light (sluggish reaction is positive) 1
. no reaction to light, haemorrhage, gross destruction (any or all of these) 2

2.5.3 Corneal lesions

Cornea (direct examination or, if necessary, with an Ultra-Violet lamp)

To determine the presence or absence of corneal opacification and to evaluate the affected area, one or two drops of 0.5% sodium fluorescein solution can be instilled into the eye (however, this must not be performed before the 24-hour reading).

If corneal opacification is difficult to determine, the eye can be examined under a UV lamp (a clear fluorescence is visible in the areas of opacification).

Opacity (degree of intensity: area most dense taken for reading)

. no ulceration or opacity 0
. scattered or diffuse areas of opacity (other than slight dulling or normal lustre), details of iris clearly visible 1
. easily discernible translucent area, details of iris slightly obscured 2
. nacrous areas, no details of iris visible, size of pupil barely discernible 3
. opaque cornea, iris not discernible through the opacity 4

Area of opacity
. one quarter (or less) but not zero 1
. greater than one quarter but less than a half 2
. greater than one half but less than three quarters 3
. greater than three quarters up to whole area. 4

Any other lesions observed were noted.

2.6 INTERPRETATION OF RESULTS AND CLASSIFICATION OF SUBSTANCES

The results obtained were evaluated in conjunction with the nature and the reversibility of the scores observed, whilst taking into account all the reactions of the treated animals.
Classification of the test substance is based on the criteria laid down in Commission Directive 93/21/EEC of 27 April 1993 adapting to technical progress for the eighteenth time Council Directive 67/548/EEC on the approximation of the laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances.

2.6.1 Interpretation of the results

Criteria for irritation
A substance or a preparation is considered irritant for the eyes if, when applied to the eye of the animal, significant severe ocular lesions are caused within 72 hours after exposure and which persist for 24 hours or more after treatment with the test substance.
All the scores at each reading time (24, 48 and 72 hours) and for an effect are used for calculating the respective mean values.

2.6.2 Classification of the test substances

- Xi symbol, indication of danger "irritant",

- phrases indicating the nature of special risks:

R 36: "Irritating to eyes"
Ocular lesions are significant if the mean score has any of the following values:
. opacity of the cornea ≥ 2, but < 3,
. lesion of the iris ≥ 1, but ≤ 1.5,
. redness of the conjunctivae ≥ 2.5,
. oedema of the conjunctivae (chemosis) ≥ 2.

Or else, if the test is performed on three animals, if at least two of them show lesions equal to one of the following values:
. opacity of the cornea ≥ 2, but < 3,
. lesion of the iris ≥ 1, but ≤ 2,
. redness of the conjunctivae ≥ 2.5,
. oedema of the conjunctivae (chemosis) ≥ 2.

R 41: "Risk of serious damage to eyes"
Ocular lesions are severe:
if the mean score has any of the following values:
. opacity of the cornea ≥ 3,
. lesion of the iris > 1.5.

Or else, if the test is performed on three animals, if at least two of them show lesions equal to one of the following values:
. opacity of the cornea ≥ 3,
. lesion of the iris = 2.

Or if they persist at the end of the observation period.

If the test substance or preparation induces irreversible colouration of the eyes, the phrase R 41 should also be applied.

2.7 PROTOCOL ADHERENCE

The study was performed in accordance with Study Protocol No. 18750 TAL and subsequent amendments, with the following deviations from the agreed Study Protocol:

. the temperature and relative humidity recorded in the animal room were sometimes outside of the target ranges specified in the protocol.

These minor deviations were not considered to compromise the validity or integrity of the study.

2.8 ARCHIVING

The study documentation and specimens generated during the course of the study are archived at CIT, 27005 Evreux, France, for 10 years after the end of the *in vivo* phase of the study.

The archived study materials include:
. protocol and possible amendments,
. raw data,
. correspondence,
. final report and possible amendments.

On completion of this period, the archived study materials will be returned to the Sponsor, or may be archived at CIT for a further period.

3. RESULTS

The observations recorded during the study are presented in table 1.

No ocular reactions were observed during the study.

Mean scores calculated for each animal over 24, 48 and 72 hours were 0.0, 0.0 and 0.0 for chemosis, 0.0, 0.0 and 0.0 for redness of the conjunctiva, 0.0, 0.0 and 0.0 for iris lesions and 0.0, 0.0 and 0.0 for corneal opacity.

4. CONCLUSION

Under our experimental conditions, the test substance [redacted] is non-irritant when administered by ocular route to rabbits.

According to the classification criteria laid down in Commission Directive 93/21/EEC (27th April 1993) adapting to technical progress for the eighteenth time Council Directive 67/548/EEC, the test substance is considered non-irritant.

Table 1: Individual ocular examinations and mean values of the scores recorded at each reading (24, 48 and 72 hours) for each animal

Rabbit number	Region of eye	Description of ocular reactions	Scores 1h D1	24h D2	48h D3	72h D4	Mean irritation score (1)	Interpretation (+) (-)
900	Conjunctivae	Chemosis	0	0	0	0	0.0	(-)
		Redness	0	0	0	0	0.0	(-)
		Discharge	0	0	0	0	0.0	
	Iris		0	0	0	0	0.0	(-)
	Corneal opacity	Intensity	0	0	0	0	0.0	(-)
		Area	0	0	0	0	0.0	
	Other Fluorescein		* /	* U	* /	* /		
47	Conjunctivae	Chemosis	0	0	0	0	0.0	(-)
		Redness	0	0	0	0	0.0	(-)
		Discharge	0	0	0	0	0.0	
	Iris		0	0	0	0	0.0	(-)
	Corneal opacity	Intensity	0	0	0	0	0.0	(-)
		Area	0	0	0	0	0.0	
	Other Fluorescein		* /	* U	* /	* /		
48	Conjunctivae	Chemosis	0	0	0	0	0.0	(-)
		Redness	0	0	0	0	0.0	(-)
		Discharge	0	0	0	0	0.0	
	Iris		0	0	0	0	0.0	(-)
	Corneal opacity	Intensity	0	0	0	0	0.0	(-)
		Area	0	0	0	0	0.0	
	Other Fluorescein		* /	* U	* /	* /		

(1) mean of scores on days 2, 3 and 4
h = hour
D = day
(+) = irritant according to E.E.C. criteria
(-) = non-irritant according to E.E.C. criteria
* = None
/ = Fluorescein not used
U = Fluorescein batch No. 7239

APPENDICES

1. Test article description and analytical certificate

TOXICOLOGY DEPARTMENT
CONFIDENTIAL
April 99

elf atochem s.a.
La défense 10, cours Michelet
92091 Paris-la-Défense, France

TEST ARTICLE DESCRIPTION

[redacted]

IDENTITY

Test article name	:	[redacted]
Chemical name	:	
CAS number	:	
EINECS number	:	
Purity	:	
Origin and batch	:	Elf Atochem, VSP
Batch	:	[redacted]
Elf Atochem filing number	:	CAL 1392/99

PHYSICAL AND CHEMICAL PROPERTIES

Appearance	:	amber liquid
pH	:	5 ± 1
Density	:	1100 kg/m^3 at 20°C
Boiling point	:	98-100 °C
Flash point	:	no flash point
Solubility	:	water : insoluble soluble in alcohols, aromatic hydrocarbons

TOXICOLOGICAL INFORMATIONS AND USE SAFETY

See safety data sheet

STORAGE AND DISPOSAL

Storage	:	in dark and at room temperature
Expiry date	:	may 2000
Disposal	:	incineration

elf atochem
ATO

Elf Atochem S.A.
Usine de Villers-Saint-Paul
ZA Villers Saint Paul - Rieux
B.P. 20 - 60870 Rieux (France)
Tél : 44.74.44.74 - Fax : 44.74.42.07 - Télex : 140 420 ATO VSP

Villers Saint Paul, le 9 Novembre 1999

CERTIFICAT D'ANALYSE [redacted]

Lot N° [redacted]	Unité	Résultat	Spécification de fabrication	Méthode d'analyse
Extrait Sec	%	30,3	29 à 31	LCU 622
Tertio Butanol	%	0,95	0 à 1	LCU 653
Acrylamide	%	0,07	0 à 0,1	LCU 658
Acide acrylique	%	0,1	0 à 2	LCU 658
Test d'extinction	/	positif	positif	SAI 021

Le Chef de Service du Laboratoire
SIGNATURE :

NOM : Robert Gruppo

Company Sanitized. Does not contain TSCA CBI

2. Diet formula

Ref: 112

COMPLETE DIET

RABBIT MAINTENANCE DIET

Appearance: 4.5 mm diameter granules

Conditioning: bags of 25 kgs

Daily portion: in accordance with race and body weight, Rabbits 100-150 g, water *ad libitum*.

FORMULA %

Cereals	43.8
Grain byproducts and legumes	49
Vegetable protein (soya bean meal, yeast)	4.2
Vitamin and mineral mixture	3

AVERAGE ANALYSIS %

Calorific value (Kcal/kg)	2200
Moisture	10
Proteins	13
Lipids	2.7
Carbohydrates (N.F.E.)	49.3
Fibre	17
Minerals (ash)	8

AMINO ACID VALUES
(calculated in mg/kg)

Arginine	6800
Cystine	2100
Lysine	4600
Methionine	1600
Tryptophan	1400
Glycine	5200

FATTY ACID VALUES
(calculated in mg/kg)

Palmitic acid	6400
Palmitoleic acid	0
Stearic acid	600
Oleic acid	6400
Linoleic acid	12100
Linolenic acid	2400

MINERALS (calculated in mg/kg)

	Nat. val.	CMV val.	Total
P	3500	3500	7000
Ca	4500	4500	9000
K	11600	0	11600
Na	400	1600	2000
Mg	2100	100	2200
Mn	40	40	80
Fe	160	140	300
Cu	12	15	27
Zn	30	45	75
Co	0.1	1.5	1.6
I	0	0	0
Cl	500	3000	3500

VITAMINS (calculated per kg)

	Nat. val.	CMV val.	Total
Vitamin A	2850 IU	6500 IU	9350 IU
Vitamin D3	30 IU	1000 IU	1030 IU
Vitamin B1	4.3 mg	0 mg	4.3 mg
Vitamin B2	3.8 mg	0 mg	3.8 mg
Vitamin B3	16 mg	0 mg	16 mg
Vitamin B6	1 mg	1 mg	2 mg
Vitamin B12	0 mg	0 mg	0 mg
Vitamin E	16 mg	10 mg	26 mg
Vitamin K3	6 mg	1 mg	7.mg
Vitamin PP	55 mg	5 mg	60 mg
Folic acid	0 mg	0 mg	0 mg
Biotin	0 mg	0 mg	0 mg
Choline	850 mg	200 mg	1050 mg
Meso-Inositol	0 mg	0 mg	0 mg

Available under quality "Control Ref.: 112 C"

UAR, 7 rue Galliéni, 91360 Villemoisson - Tel : 01.69.04.03.57 - Fax : 01.69.04.81.97
(Ref. Doc. UAR : 1992)